

## Phytoremediation of zinc and copper by using *Acacia mangium* plant in laboratory contaminated soils

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### ABSTRACT

The following study presents the effects of Ethylenediaminetetraacetic acid (EDTA), biochar, and humic acid on zinc and copper uptake in plant tissues. Treatments were conducted successively in an arrangement that the main consideration was towards metal uptake in root and shoot tissues by keeping the properties of chelation which is developed through EDTA and enhanced capabilities in the soil performance of biochar and humic acid. These results indicated that EDTA at 5 µg/kg significantly increased Zn accumulation in the roots at  $861.361 \pm 1188.849$  µg/kg,  $p = 0.033$ , higher than that of both biochar and humic acid treatments combined. Among root tissues, Cu accumulation was also highest in EDTA treated at 2.5 µg/kg, amounting to  $594.649 \pm 836.200$  µg/kg,  $p = 0.034$ . Most noticeably, humic acid at 10 µg/kg increased Cu uptake in the roots at  $3109.425 \pm 7187.589$  µg/kg,  $p = 0.017$ , thus may have acted to increase metal availability. Shoot metal accumulation did not differ significantly across treatments, however. These results point to the effectiveness of EDTA in enhancing the root uptake of metals while biochar and humic acid generally produced small but differential effects. The findings underline the importance of choosing a treatment for metals uptake optimization in plants and enlighten the possible use of soil amendments for better nutrient management.

**Keywords:** phytoremediation, heavy metals, *acacia mangium*, EDTA, biochar, humic acid.

### INTRODUCTION

Soil contamination with heavy metals like Zn and Cu is one of the major environmental issues the entire world faces. Even though those metals are essential micronutrients for plant growth, their higher concentrations beyond threshold levels have been found to be toxic (Angon *et al.*, 2024). It is normally caused by industrial activities, mining operations, and poor waste management practices that degrade the quality of the soil and hence pose potential risks to human health and ecosystems (Simion, *et al.*, 2021). The temporal trends in precipitation were weaker than those in temperature (Abdaki *et al.*, 2024). This problem needs the application of sustainable, cost-effective, environmentally benign remediation techniques. Among them, phytoremediation involves the use of plants to remove, stabilize, or degrade contaminants in soils (Sharma *et al.*,

2022). Among such phytoremediation plants, *Acacia mangium* has been emerging as one of the promising candidates since it grows fast, can produce very high biomass, and is adapted to nutrient-poor and metal-contaminated soils (Bongoua-Devisme *et al.*, 2019).

*Acacia mangium* is a leguminous tree originally distributed in Australia, Papua New Guinea, and Indonesia. It has been widely recognized as one of the forest species that can grow in degraded environments (Koutika and Richardson, 2019). This had a robust root system with the symbiosis of nitrogen-fixing bacteria, hence increasing the fertility of the soil to some extent for better heavy metals uptake. Large-sized plant roots might extract metals even from deeper layers of soil, while above-ground biomass is usually the major sink for metal accumulation (Avani, *et al.*, 2014). That makes *Acacia mangium* a

good candidate for the phytoremediation of Zn and Cu-contaminated soils (Zuhaidi, 2018).

Zn and Cu are among the most prevalent heavy metal contaminants in soils (Iglikowska *et al.*, 2024). In spite of the fact that Zn is considered an essential micronutrient for enzymatic functions and plant development, its high concentrations induce phytotoxicity expressed by stunted growth, chlorosis, and lower crop yields (Balafrej *et al.*, 2020). Cu is an essential element involved in photosynthesis and protein synthesis, but higher concentrations induce toxicity by oxidative stress and subsequently lead to cell damage (Chen *et al.*, 2022). Bioaccumulation of metals exerts harmful effects that may be mitigated in phytoremediation with *Acacia mangium* in a greener way (Zuraidi, *et al.*, 2018) metals within the plant tissues decrease the bioavailability in soil.

Phytoremediation consists of phytoextraction, phytostabilization, phytodegradation, and rhizofiltration. Phytoextraction has been defined as the uptake and translocation of heavy metals to the aerial part of the plant, which can be harvested later to get the contaminants removed from the site (Tan *et al.*, 2023). On the other hand, phytostabilization refers to immobilizing the heavy metals and preventing them from leaching into groundwater or their uptake by other plants (Nedjimi, 2021). In relation to the *Acacia mangium* species, high phytoextraction and phytostabilization potentials are advantageous in the management of zinc and copper contamination (Bongoua-Devisme *et al.*, 2019). These are supported by laboratory experiments on the efficiency of *Acacia mangium* in accumulating heavy metals from contaminated soils. Experimental setup simulates the real contamination by addition of controlled concentrations of zinc and copper into the soil samples (Riza and Hoque, 2021). Growth responses of *Acacia mangium*, with metal concentration in root, stem, and leaf, give insight into the phytoremediation potential of the plant. The uptake and accumulation of heavy metals are influenced by soil pH, organic matter content, and metal speciation; hence, optimizing growth conditions with respect to maximum remediation efficiency becomes important (Cambier *et al.*, 2014).

Concentration Besides its use in phytoremediation, *Acacia mangium* can improve soil structure, build up organic matter, and increase biodiversity in ecosystem restoration. The fact that it can resist high metal concentration without the development of striking toxicity symptoms

speaks for its resilience and suitability for large-scale remediation projects. Besides ecological, the economic benefits of the use of *Acacia mangium* in phytoremediation are also important. The biomass have harvested and can be used for the further production of bioenergy, pulp and paper, or as feedstock for biochar. This contributes to a land reclamation approach based on the principles of a circular economy (Koutika and Richardson, 2019).

Nevertheless, these are the pros; in fact, phytoremediation with *Acacia mangium* has some drawbacks. This method generally is slower compared to the other methods of remediations involving excavation of soil and chemicals (Hasan *et al.*, 2019). In addition, phytoremediation works properly with bioavailability of heavy metals dependent on the type of soil and surrounding environmental conditions. The approaches for the improvement of these challenges are inter-multi-disciplinary approaches of agronomy, soil science, and environmental engineering (Wei *et al.*, 2021).

However, it is, in any case, in the right direction for the effective phytoremediation of zinc and copper-contaminated soils by *A. mangium* with higher ends in view like sustainable land management against environmental pollution (Nedjimi, 2021). In view of challenges at the level of struggles that the nation-state faces with the SDGs, phytoremediation remains imperative, especially with Goal 15, Life on Land, and Goal 13, Climate Action, not only for the rejuvenation of degraded lands but also for restoring negative impacts within heavy metal-contaminated sites (Arora and Mishra, 2023; Kumar and Isha, 2024).

One of the many feasible methods for this ever-growing problem is employing *Acacia mangium* on zinc and copper contaminated soils for phytoremediation. *Acacia mangium* will provide a continued, economic, and environmentally harmless method for soil remediation, considering the plant's distinct physiological traits and ecological advantages (Sharma *et al.*, 2023). Its full potentials, however, need further research and field trials for healthier ecosystems and thus more resilient agricultural landscapes.

While various plant species have been studied for their phytoremediation potential, the focus of this research is on *Acacia mangium*, a hardy and fast-growing tree species known for its adaptability to diverse environmental conditions. *Acacia mangium* has shown promise

in previous studies as a potential candidate for phytoremediation due to its robust root system and metal-tolerant characteristics.

Despite the growing body of literature on phytoremediation, there is a noticeable gap in research pertaining to the application of *Acacia mangium* for the remediation of zinc and copper-contaminated soil, particularly in the context of Mosul city, Iraq. The unique environmental conditions and the prevalence of heavy metal contamination in Mosul city underscore the need for region-specific studies to develop effective and sustainable remediation strategies.

The current study aims to address this knowledge gap by investigating the phytoremediation capabilities of *Acacia mangium* in laboratory-contaminated soil with zinc and copper. Through a systematic examination of the plant's uptake mechanisms and its impact on metal concentrations in soil, this research contributes to the broader understanding of phytoremediation strategies tailored to the environmental challenges faced by Mosul city.

## MATERIAL AND METHODS

The samples of soil were taken from the non-contaminated area and sieved to discard big debris. Afterwards, the samples were air-dried at room temperature for 48 hours and homogenized (Liang *et al.*, 2025). To simulate contamination, solutions of Zn and Cu were prepared by the dissolution of  $\text{ZnSO}_4$  and  $\text{CuSO}_4$  salts in deionized water. Further, these solutions were added to the soil at concentrations of 2.5, 5, and 10  $\mu\text{g/kg}$  with proper mixing to ensure homogeneous contamination (Albanna *et al.*, 2022).

The experiment was a completely randomized design (CRD) with three replicates per each treatment group (García *et al.*, 2014). This approach is a basic experimental design in which all experimental units are assigned to different treatments completely at random (Dafaallah, 2019). The *Acacia mangium* seedlings, each in pots containing 3 kg of the contaminated soil, were treated with the following amendments: EDTA (ethylene diamine tetraacetic acid), Biochar and Humic acid. Each amendment was applied at the concentration level of 2.5, 5, and 10  $\mu\text{g/kg}$  in order to find the efficiency of these chelating agents in enhancing the uptake and accumulation of Zn and Cu by *Acacia mangium*.

Seeds of *Acacia mangium* were directly germinated in nursery trays and later transferred to experimental pots after three weeks. The seedlings were grown under controlled greenhouse conditions: temperature  $25 \pm 2^\circ\text{C}$ , humidity 60%, and light with a 16-hour photoperiod. Plants were irrigated every two days using deionized water, maintaining the soil moisture at 70% of field capacity (Oreggioni *et al.*, 2019).

Plants were harvested after 90 days of growth. The plants were separated into shoots and roots, washed thoroughly with de-ionized water, and oven-dried at  $70^\circ\text{C}$  for 72 h, until constant weight was achieved. The dried plant material was ground to a fine powder for further analysis. After that, the fine powder is digested by several acids.

Atomic absorption spectroscopy (AAS) was used for the measurements of Zn and Cu concentrations in shoot and root of the tissues. About 0.5 g of powder was digested with the mixture  $\text{HNO}_3 : \text{HCl}$  (3 : 1) in microwave digestion system. The filtrate from digested samples filtered and then diluted to 50 mL with deionized water and analyzed (Paul and Pandey, 2024).

The metal accumulation in plant tissues was expressed as mean  $\pm$  S.D. for each treatment group. Data was analyzed by one-way analysis of variance (ANOVA) for significant difference among treatments (Webster and Lark, 2018). Pairwise differences were detected by Tukey post-hoc test. The level of statistical significance was set at  $p < 0.05$ .

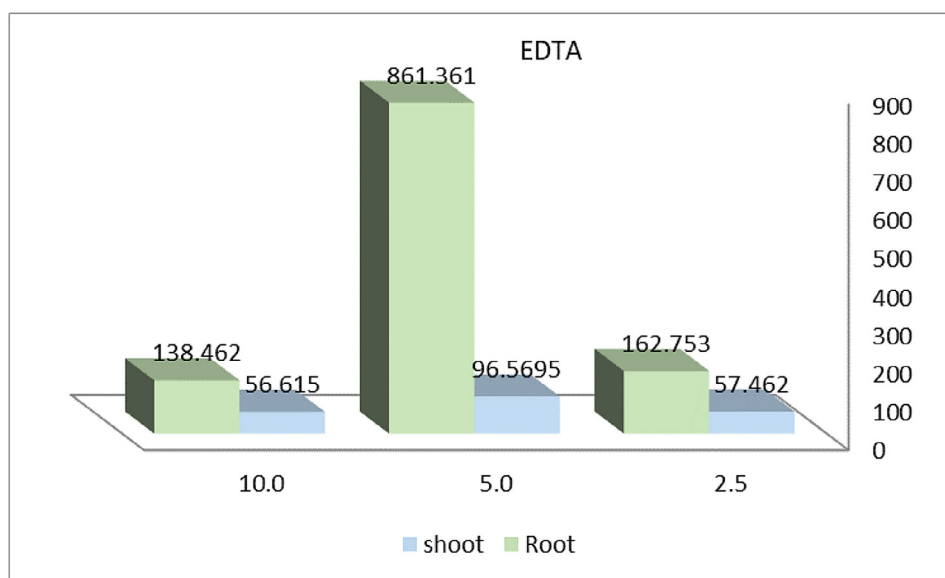
## RESULTS

The results showed that there were significant differences in Zn accumulation among the treatments. In the root tissues, 5  $\mu\text{g/kg}$  EDTA had the highest zinc uptake, amounting to  $861.361 \pm 1188.849 \mu\text{g/kg}$ , which was statistically significant ( $p = 0.033$ ) when compared with other treatments (Table 1). The 2.5  $\mu\text{g/kg}$  EDTA treatment also caused a relatively lower but still significant zinc accumulation in the roots at  $162.753 \pm 231.556 \mu\text{g/kg}$  (Figure 1).

In contrast, both the treatments of biochar and humic acid showed relatively low values of zinc accumulation in the roots. For biochar treatments, the value range was within  $141.892 \pm 184.687 \mu\text{g/kg}$  (2.5  $\mu\text{g/kg}$ ) and  $226.721 \pm 286.881 \mu\text{g/kg}$  (5.0  $\mu\text{g/kg}$ ), while humic acid treatments showed values in a range between

**Table 1.** Accumulation of zinc in root and shoot tissues

Treatment	Zn in shoots ( $\mu\text{g/kg}$ )	Zn in roots ( $\mu\text{g/kg}$ )	F-value	p-value
EDTA 2.5	$57.462 \pm 53.579$	$162.753 \pm 231.556$	4.668	0.033*
EDTA 5.0	$96.569 \pm 106.987$	$861.361 \pm 1188.849$		
EDTA 10.0	$56.615 \pm 68.642$	$138.462 \pm 171.064$		
Biochar 2.5	$40.266 \pm 46.732$	$141.892 \pm 184.687$	0.411	0.668
Biochar 5.0	$24.358 \pm 24.389$	$226.721 \pm 286.881$		
Biochar 10.0	$26.997 \pm 34.774$	$207.611 \pm 368.318$		
Humic 2.5	$33.727 \pm 29.570$	$183.788 \pm 401.336$	3.860	0.042*
Humic 5.0	$16.594 \pm 10.569$	$254.254 \pm 382.259$		
Humic 10.0	$7.939 \pm 3.6115$	$180.364 \pm 259.585$		

**Figure 1.** Histogram comparison ability of *Acacia mangium* plant for Zn levels after treatment of EDTA with various concentrations (2.5, 5, 10  $\mu\text{g}$ ), between shoot and root

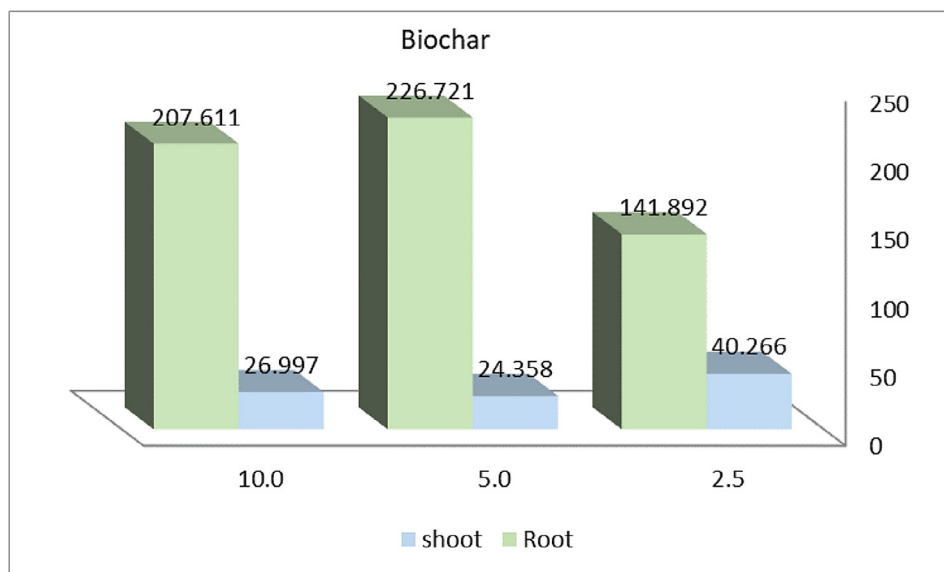
$183.788 \pm 401.336 \mu\text{g/kg}$  (2.5  $\mu\text{g/kg}$ ) and  $254.254 \pm 382.259 \mu\text{g/kg}$  (5.0  $\mu\text{g/kg}$ ) (Figure 2).

Among shoot tissues, EDTA at 5  $\mu\text{g/kg}$  presented the highest accumulation of zinc,  $96.569 \pm 106.987 \mu\text{g/kg}$ , though not significant,  $p > 0.05$ , showing that there was little variation in the uptake of zinc in the shoot tissues among the treatments. In general, the shoot zinc levels from the biochar and humic acid treatments were generally low; for the application of biochar, they ranged between  $24.358 \pm 24.389 \mu\text{g/kg}$  and  $40.266 \pm 46.732 \mu\text{g/kg}$  at 5.0 and 2.5  $\mu\text{g/kg}$ , respectively, while for humic acid, between  $7.939 \pm 3.6115 \mu\text{g/kg}$  of 10  $\mu\text{g/kg}$  and  $33.727 \pm 29.570 \mu\text{g/kg}$  of 2.5  $\mu\text{g/kg}$ .

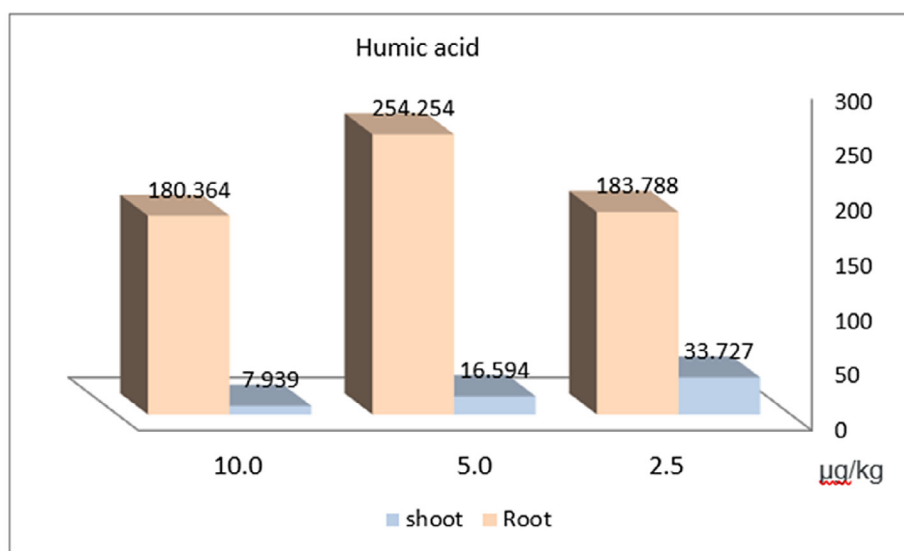
In short, 5  $\mu\text{g/kg}$  EDTA was the most effective treatment to enhance zinc accumulation in root tissues, and the shoot zinc accumulation did not differ significantly among treatments.

In contrast, Copper accumulation followed patterns similar to those of zinc accumulation in that the highest, significantly different, copper uptake with EDTA application of 2.5  $\mu\text{g/kg}$  was  $594.649 \pm 836.200 \mu\text{g/kg}$ , with a  $p\text{-value} = 0.034$  (Table 2). Copper increase was also provided to root tissues by application with 5  $\mu\text{g/kg}$  EDTA ( $215.224 \pm 457.193 \mu\text{g/kg}$ ), with the variation not being significantly different with regard to the other test and control samples ( $p > 0.05$ ). On the other hand, EDTA at 10  $\mu\text{g/kg}$  showed significantly lower copper accumulation in the roots at  $65.973 \pm 41.316 \mu\text{g/kg}$ , showing that the higher the EDTA concentration, the lesser the copper uptake in a concentration-dependent manner (Figure 3).

In shoot tissues, EDTA at 5  $\mu\text{g/kg}$  resulted in the highest accumulation of copper, which was



A



B

**Figure 2.** Histogram comparison ability of *Acacia mangium* plant for Zn levels after treatment of biochar & humic acid with various concentrations (2.5, 5, 10 µg), between shoot and root, (A) Comparative histogram showing zinc (Zn) accumulation in the roots and shoots of *Acacia mangium* treated with biochar at varying concentrations (2.5, 5, and 10 µg), (B) Comparative histogram showing zinc (Zn) accumulation in the roots and shoots of *Acacia mangium* treated with humic acid at varying concentrations (2.5, 5, and 10 µg)

136.127 ± 222.133 µg/kg, and was significantly different, with a p-value of 0.036. However, the copper levels were generally low in shoots across all the treatments of biochar and humic acid. In the case of copper accumulation, the ranges in shoots for the biochar treatments varied from 12.906 ± 9.351 µg/kg to 26.997 ± 34.774 µg/kg, corresponding to the 2.5 µg/kg and 10 µg/kg

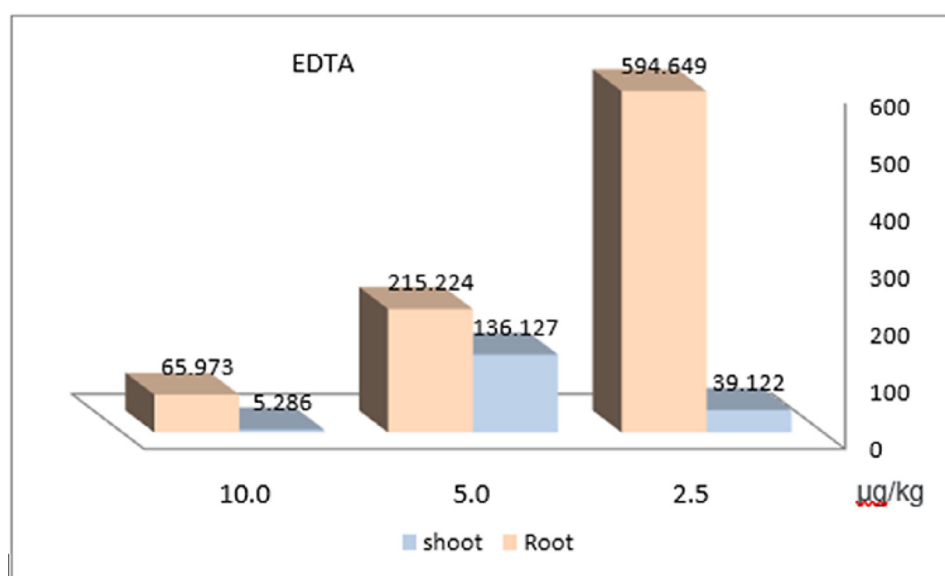
biochar treatments, respectively, and for humic acid, from 7.939 ± 3.6115 µg/kg at 10 µg/kg to 33.727 ± 29.570 µg/kg at 2.5 µg/kg, showing no significant differences (Figure 4).

The humic acid at 10 µg/kg has copper accumulation in the root tissues that is significantly higher (3109.425 ± 7187.589 µg/kg, with a p-value of 0.017), suggesting its ability to stimulate



**Table 2.** Accumulation of copper in root and shoot tissues

Treatment	Cu in shoots ( $\mu\text{g/kg}$ )	Cu in roots ( $\mu\text{g/kg}$ )	F-value	p-value
EDTA 2.5	$39.122 \pm 51.111$	$594.649 \pm 836.200$	6.700	0.036*
EDTA 5.0	$136.127 \pm 222.133$	$215.224 \pm 457.193$		
EDTA 10.0	$5.286 \pm 1.33712$	$65.973 \pm 41.316$		
Biochar 2.5	$12.906 \pm 9.351$	$1043.064 \pm 1877.487$	0.366	0.698
Biochar 5.0	$24.358 \pm 24.389$	$226.721 \pm 286.881$		
Biochar 10.0	$26.997 \pm 34.774$	$207.611 \pm 368.318$		
Humic 2.5	$33.727 \pm 29.570$	$183.788 \pm 401.336$	3.860	0.042*
Humic 5.0	$16.594 \pm 10.569$	$254.254 \pm 382.259$		
Humic 10.0	$7.939 \pm 3.6115$	$180.364 \pm 259.585$		

**Figure 3.** Histogram comparison ability of *Acacia mangium* plant for copper levels after treatment of EDTA with various concentrations (2.5, 5, 10  $\mu\text{g}$ ), between shoot and root

higher copper uptakes in the roots than other treatments at higher concentrations.

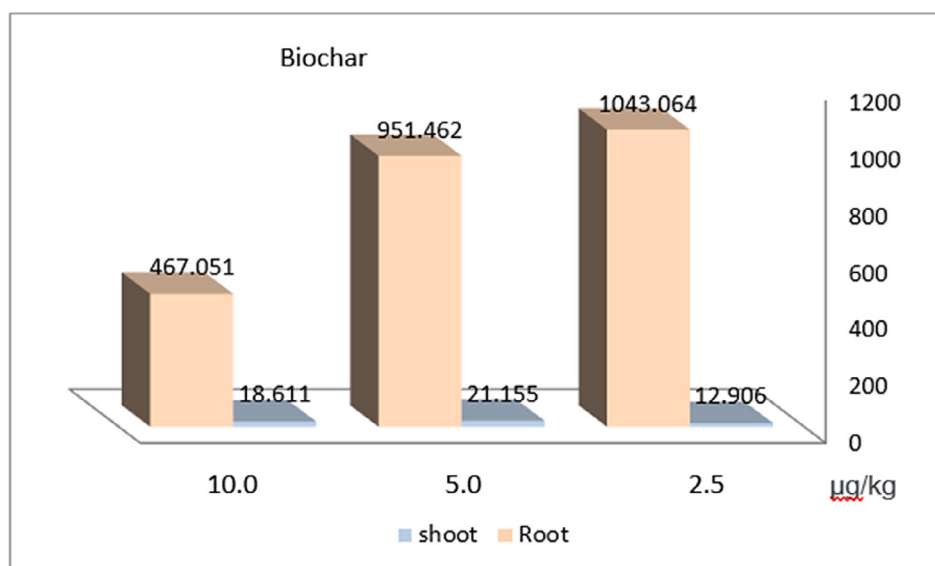
Overall, EDTA was the best treatment for enhancing copper accumulation in roots at concentration 2.5  $\mu\text{g/kg}$ . While, at 5  $\mu\text{g/kg}$  EDTA caused the highest copper uptake in shoots. Humic acid at 10  $\mu\text{g/kg}$  was particularly effective for copper uptake in the roots, though it did not affect the copper accumulation in shoots noticeably.

One-way ANOVA analysis revealed significant differences in metal accumulation across treatments. In the case of zinc accumulation in roots, the F-value of 4.668 and a p-value of 0.033 showed significant variation between treatments (Table 1). For copper accumulation in roots, the F-value of 6.700 and a p-value of 0.036 showed significant differences in copper uptake across the treatments (Table 2). All the p-values were

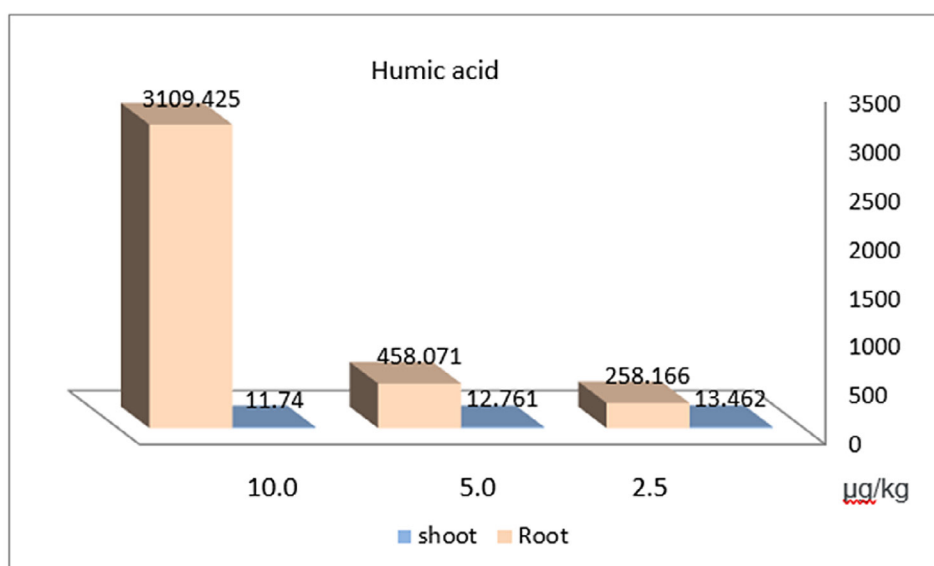
generally greater than 0.05 in shoot tissues so that the treatments could not significantly influence metal accumulation in the shoot. This showed that applications of EDTA at 2.5 and 5  $\mu\text{g/kg}$  considerably increased uptakes of zinc and copper in the root, while treatments involving biochar and humic acid showed less effect on metals.

## DISCUSSION

Present research work was carried out to investigate the Zn and Cu uptake in plant tissues subjected to different treatments with EDTA, biochar, and humic acid. Generally, the trend of metal uptake showed a wide variation due to the treatments applied in general; EDTA showed more extensive influences, especially at particular



A



B

**Figure 4.** Histogram comparison ability of *Acacia mangium* plant for copper levels after treatment of biochar & humic acid with various concentrations (2.5, 5, 10 µg), between shoot and root, (A) Comparison of copper accumulation in shoots and roots of *Acacia mangium* under different concentrations (2.5, 5, and 10 µg) of biochar treatments, presented as a histogram, (B) Comparison of copper accumulation in shoots and roots of *Acacia mangium* under different concentrations (2.5, 5, and 10 µg) of humic acid treatments, presented as a histogram

concentrations, when compared with biochar and humic acid, showing very minor influences on the processes of metals accumulation.

Regarding zinc accumulation, EDTA at 5 µg/kg gave the highest uptake in root tissues,

which was  $861.361 \pm 1188.849$  µg/kg, significantly outperforming the other treatments. This result corroborates the fact that EDTA is an established chelating agent that increases the bio-availability of metals in the soil, thus promoting

their uptake by plant roots (Yin et al., 2024). The large increase in root Zn accumulation following EDTA treatment suggests that chelation of zinc ions by EDTA may have rendered it more available to the plant and enhanced its uptake by roots. Interestingly, no significant differences were found in shoot Zn accumulation among the treatments ( $p > 0.05$ ), which may mean that while EDTA enhances root uptake, the efficiency of translocation or other storage mechanisms within the shoot are less affected by the applied treatments.

Biochar and humic acid treatments resulted in relatively lower levels of Zn accumulation than in EDTA. Biochar, a carbon-rich material, has been shown to improve nutrient retention and water-holding capacity in soils (Lehmann et al., 2011), but its effect on Zn uptake appears less pronounced in this study. The lower zinc accumulation observed in biochar-treated plants could be attributed to the sorption properties of biochar, which might reduce the mobility of Zn in the soil or restrict its availability to plant roots. Similarly, humic acid, usually used to enhance soil fertility and microbial activity, by Khaleel et al. (2015), resulted in relatively less increase in Zn accumulation, which could be attributed to the complex interactions with the soil constituents themselves and nutrient uptake promotion due to improvement of soil structure rather than through direct metal chelation.

Patterns of copper accumulation were similar to the one observed for zinc: copper uptake was higher in roots and it showed a maximum in EDTA 2.5  $\mu\text{g/kg}$ , namely,  $594.649 \pm 836.200 \mu\text{g/kg}$ , which suggests that EDTA ability of solubilizing metals through chelation strongly enhanced metal uptake from plant roots, according to the hypothesis Puschenreiter et al. (2005). In shoots, the highest copper accumulation for EDTA at 5  $\text{mg/kg}$  showed a similar trend when in the roots, supporting EDTA in enhancing copper translocation from the roots to the shoot. Higher copper accumulation, as found in lower concentrations of EDTA, reveals a concentration-dependent effect by EDTA, which, as observed in other experiments, might be explained because high EDTA concentrations eventually inhibit the uptake of the metals through competitive interactions and hence its toxicity (Zand and Mühling, 2022).

Especially, the treatment of humic acid at 10  $\mu\text{g/kg}$  proved to be most effective in increasing

copper accumulation in root tissues, exhibiting a value of  $3109.425 \pm 7187.589 \mu\text{g/kg}$ , which is new. The humic substances interact with metal ions, thereby enhancing their availability in soils (Ghosh et al., 2017). The much higher magnitude in copper accumulation in the roots under the humic acid treatment might be because of an increase in soil-plant metal interaction. Therefore, humic acid most probably increased the uptake of copper by increasing the surface area of the root and enhancing the mobility of metals in the rhizosphere.

Overall, this study quantified the differential effects of EDTA, biochar, and humic acid on metal accumulation. EDTA, especially 5  $\mu\text{g/kg}$  zinc and 2.5  $\mu\text{g/kg}$  copper, proved to be the best treatment in the case of improving metal uptake, especially in root tissues. Biochar and humic acid caused some effect on metal accumulation but proved less effective than EDTA. These results suggest that EDTA was the most efficient chelating agent to enhance metal availability and uptake, whereas biochar and humic acid may act complementarily in improving soil structural properties and overall nutrient availability. Long-term experiments are necessary to investigate effects of those treatments on plant health and metal bioavailability and also the environmental effects (Edrees et al., 2022).

## CONCLUSIONS

This study demonstrated the potential of *Acacia mangium* in the phytoremediation of zinc (Zn) and copper (Cu) from contaminated soil using different soil amendments. The finding confirmed that EDTA at a concentration of 5  $\mu\text{g/kg}$  significantly enhanced the accumulation of Zn in the roots of *Acia mangium*, surpassing the effects of other amendments such as humic acid and biochar. In contrast, the highest accumulation of Cu was observed in roots treated with EDTA at 2.5  $\mu\text{g/kg}$  concentration, while humic acid at 10  $\mu\text{g/kg}$  concentration also contributed to increasing Cu uptake. These findings highlight the role of specific chelating agents and their concentration in influencing heavy metal uptake. *A. manguium* combined with tailored amendments shows promise for phytoremediation of Zn and Cu-contaminated soils.



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